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I. J. Lipkin

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ON THE DISTRIBUTION AND DESTRUCTION OF QUININE IN ANIMAL TISSUES

BY

I. J. LIPKIN

M.D., Ch.B., M.R.C.S., L.R.C.P., D.P.H., D.T.M.

JOHNSTON FELLOW IN BIO-CHEMISTRY, UNIVERSITY OF LIVERPOOL

From the Department of Bio-chemistry, University of Liverpool

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INTRODUCTION

In a recent paper by Ramsden, Lipkin, and Whitley (1918), it was shown that 'Quinine introduced into an animal in large doses accumulates in most of the tissues at very much higher concentrations than in the blood,' and also that 'the liver of rabbits, guinea-pigs, oxen and sheep rapidly attacks quinine post mortem.'

It was shown also (p. 256) that even a concentration of 16·6 mgm. of quinine per litre, of blood, a concentration so high as to be almost intolerable to the patient, and which had been maintained at this height for at least thirty-three hours, nevertheless failed to effect a radical cure in a case of malaria.

The experiments recorded in the present paper continue the investigation, and are concerned mainly with

1. The distribution of ingested quinine in animal tissues.
2. The power of various tissues to destroy quinine.
3. The nature of the quinine-destroying agent and the conditions favouring its activity.
4. The substances resulting from such destruction of quinine.

DISTRIBUTION OF INGESTED QUININE

The methods used for the extraction of the quinine from tissues are those described by Ramsden and Lipkin in previous papers (1918*a*, 1918*b*).

1. *The Ammonium Sulphate Method*, serviceable for most tissues, e.g., spleen, kidney, suprarenal, muscle, bone marrow, testis.

I have now tested this method with salivary and thyroid glands and intestinal wall, and find it equally reliable.

Tissue	Animal	Weight of tissue grms.	Quinine given mgms.	Quinine found mgm.
Thyroid	Sheep	10	0·5	0·5
(Submaxillary) Salivary gland	„	7·5	0·5	0·5

NOTE.—(1) In the estimation of quinine in the walls of the large and small intestine it was found desirable to treat the ammonium sulphate filtrate with 5% of its volume of 25% lead acetate solution and filter before extraction with ether, thereby removing certain impurities which themselves give Tanret turbidity; (2) Losses of less than 5% are to be regarded as within the range of experimental error.

About 5 grms. of the tissue is transferred direct from the animal into a weighed flask containing about 10 c.c. of a saturated aqueous solution of $(NH_4)_2SO_4$ + 0·6 per cent. H_2SO_4. The flask is re-weighed, then boiled for two minutes, and its contents filtered under pressure through a Gooch crucible or a Buchner filter.

The residue is pulped in a mortar with glass-powder and again boiled up in the original flask with successive lots of acidulated $(NH_4)_2SO_4$ solution. The combined filtrates are shaken with ether to extract 'oily' matter, then alkalised strongly with ammonia and again shaken up with ether to extract the quinine. Evaporate each lot of ether as it separates in one of the tubes guaged for nephelometry. Dissolve the residual quinine by boiling it with a sufficiency of saturated ammonium sulphate and estimate nephelometrically against quinine standards.

2. *The alcohol extraction method for brain and fat.*

Weigh out about 10 grammes of tissue, grind up to a fine pulp with powdered glass, and transfer it to a flask with the aid of boiling absolute alcohol.

Boil and filter into a graduated cylinder. Repeat the boiling and filtering with three further lots of alcohol. Note volume of filtrate, pour it into five times its volume of 1 per cent. H_2SO_4, and shake thoroughly for five minutes. Extract the fats and lipoids by shaking with three successive lots of pure ether. For every 100 c.c. of fat-free liquid add 5 c.c. of 25 per cent. Pb. acetate solution, filter off aliquot portion of the whole into a stoppered cylinder. Saturate with

$(NH_4)_2SO_4$, pipette off the layer of alcohol-ether which separates, and again extract with ether until the extracts are colourless. Alkalise with NH_4OH, extract quinine with four successive lots of ether, evaporating each lot as separated in one of the tubes gauged for nephelometry. Dissolve the residual quinine by boiling it with a sufficiency of known volume of saturated aqueous ammonium sulphate and estimate nephelometrically.

EXPERIMENT I. Guinea-pig, 755 grammes in weight. 0·4 grammes of quinine dissolved in 10 c.c. of water with enough HCl to effect its solution was injected into the peritoneal cavity. The solution was not acid to Congo red, or methyl orange, although acid to litmus, and its osmotic pressure was calculated as about half that of 0·9 per cent. NaCl. The animal died fifteen minutes after the injection. The peritoneal cavity, which was in parts denuded of its epithelial lining, contained 5 c.c. of turbid faintly yellow fluid full of leucocytes and masses of rolled-up epithelial cells. Each organ investigated was rinsed free from peritoneal fluid by salt solution and wiped dry before weighing. Blood was taken from the left ventricle, urine from the bladder. The intestines were congested, but nothing else abnormal was noticeable. There were no notable symptoms during the period between the injection and the death of the animal.

Tissue	Grammes of tissue taken	Mgm. quinine found	Mgm. quinine per 100 grammes of tissue
Suprarenal	0·35	4·23	1210·0
Peritoneal Fluid	2·15	14·6	679·0
Kidney	2·27	1·93	85·0
Spleen	0·75	0·285	38·0
Liver	3·25	1·21	37·5
Pancreas	1·53	0·23	15·0
Muscle	2·30	0·20	9·0
Blood	1·15	0·055	4·73
Brain	2·68	0·065	2·86
Urine	2·3 c.c.	0·06	2·6 per 100 c.c.

Hence in fifteen minutes 91 per cent. of the quinine had been absorbed. The very high quinine content of the suprarenal confirms the previous results of Ramsden, Lipkin and Whitley (1918), and this notwithstanding the probability that, owing to the short interval elapsing before death, quinine accumulation had not reached its maximum.

EXPERIMENT II. Guinea-pig, 463 grammes in weight. 0·4 grammes of quinine dissolved in 2·5 c.c. of water, with enough HCl to effect its solution, was injected into the gluteal muscle. The injected fluid was not acid to Congo red. The animal showed great restlessness after fifteen minutes, and was killed by a blow on the occiput seventy-five minutes after the injection. The site of the injection showed nothing remarkable, and no fluid or exudate could be found there.

Tissue	Grammes of tissue taken	Mgm. quinine found	Mgm. quinine per 100 grammes of tissue
Suprarenal	0·22	0·05	25·25
Thyroid	0·10	0·025	25·0
Spleen	0·3	0·068	22·9
Bone marrow	0·07	0·009	13·0
Small intestine (wall)	0·30	0·037	12·35
Kidney	3·10	0·26	8·3
Brain	2·44	0·14	5·9
Large intestine (wall)	2·02	0·09	4·8
Blood	3·84	0·14	3·7
Muscle	4·9	0·14	2·73
Heart muscle	1·17	0·029	2·5
Liver	1·82	0·04	2·5
Bile	2·4 c.c.	0·006	0·25 per 100 c.c.

Although the suprarenals have again the highest percentage of quinine, their lead is small, and the concentration is very much less than with intraperitoneal injections.

EXPERIMENT III. Buck rabbit, weight 1380 grammes. 1250 mgm. quinine dissolved in 5 c.c. of water with just enough HCl to effect its solution was injected into the gluteal muscle of the leg. The fluid injected was not acid to Congo red. The animal died in convulsions in fifteen minutes.

Tissue	Grammes of tissue taken	Mgm. quinine found	Mgm. quinine per 100 grammes of tissue
Suprarenal	0·21	0·062	29·76
Kidney	4·24	1·25	29·5
Large intestine (wall)	0·32	0·05	17·4
Small intestine (wall)	0·48	0·08	16·66
Spleen	0·32	0·036	11·16
Bone marrow	0·37	0·03	8·3
Muscle	1·33	0·102	7·7
Brain	2·65	0·12	4·54
Liver	2·35	0·09	3·85
Testes	0·78	0·02	3·03
Blood	6·87	0·08	1·17

Urine turbid with phosphates. Quinine, blood and albumen absent.

The short time elapsing before death was probably not long enough to permit of the differences of distribution in the various tissues attaining their maximum.

EXPERIMENT IV. Buck rabbit, weight 1410 grammes. 0·500 mgm. quinine in 1 c.c. of water with enough HCl to effect its solution injected into the gluteal region. The animal died seventy minutes after the injection.

Tissue	Grammes of tissue taken	Mgm. quinine found	Mgm. quinine per 100 grammes of tissue
Suprarenal	0·25	0·07	28·6
Spleen	0·24	0·057	24·0
Kidney	4·07	0·80	20·0
Lung	0·57	0·06	11·16
Fat	0·52	0·058	11·1
Marrow	0·645	0·05	8·6
Muscle	1·20	0·08	7·0
Heart muscle	1·23	0·066	5·4
Large intestine (wall)	1·88	0·086	4·6
Liver	3·35	0·9	2·8
Brain	2·51	0·067	2·7
Small intestine (wall)	1·40	0·037	2·7
Testes	1·36	0·028	2·1
Stomach	3·89	0·047	1·23
Lymph gland	0·68	0·006	0·9
Blood arterial	8·43	0·101	1·2
Blood venous	4·71	0·068	1·46

Venous blood was taken from the iliac vein on the side of the injection; arterial blood from the left ventricle. It is noteworthy that the venous blood coming from the side of injection is richer in quinine than the arterial blood from the heart. The lymph glands examined were from the cervical chain. The site of the injection showed nothing abnormal.

EXPERIMENT V. Guinea-pig, weight 685 grammes. 400 mgm. quinine in 2 c.c. of solution injected into the gluteal muscle. Animal killed sixty minutes later.

Tissue	Grammes of tissue taken	Mgm. quinine found	Mgm. quinine per 100 grammes of tissue
Suprarenal	0·25	0·09	38·0
Spleen	0·63	0·15	24·0
Small intestine (wall)	0·45	0·06	14·6
Bone marrow	0·68	0·08	12·6
Kidney	2·46	0·21	8·8
Large intestine (wall)	0·64	0·03	5·3
Brain	2·54	0·12	4·8
Blood	5·85	0·15	2·6
Fat	1·32	0·03	2·3
Liver	6·44	0·14	2·3
Muscle	2·45	0·056	2·3
Lymph gland	0·64	0·005	0·8

It should be noted that (*a*) no haemolysis was found even in the animals poisoned with quinine, (*b*) quinine rapidly disappeared from the site of injection.

The following table furnishes a conspectus of the accumulation observed in the different tissues:—

	Intramuscular Injections								Intraperitoneal Injection	
Animal	Guinea-pig		Guinea-pig		Buck rabbit		Buck rabbit		Guinea-pig	
Mgms. Q. per 100 grammes body weight	86·4		58·4		35·4		90·6		53	
Time after injection	75 min.		60 min.		70 min.		15 min.		15 min.	
Tissue	Mgm. Q. per 100 grammes of tissue	Tissue Q. / Blood Q.	Mgm. Q. per 100 grammes of tissue	Tissue Q. / Blood Q.	Mgm. Q. per 100 grammes of tissue	Tissue Q. / Blood Q.	Mgm. Q. per 100 grammes of tissue	Tissue Q. / Blood Q.	Mgm. Q. per 100 grammes of tissue	Tissue Q / Blood Q.
Blood	3·7	—	2·6	—	1·2	—	1·177	—	4·73	—
Suprarenal	25·25	7·0	38·0	14·6	28·6	23·83	29·76	25·8	1210·0	256
Spleen	22·9	6·0	24·0	9·23	24·0	20·0	11·16	9·7	38·0	8
Kidney	8·3	2·3	8·8	3·4	20·0	16·66	29·5	25·5	85·0	18
Liver	2·5	0·7	2·3	0·88	2·8	2·33	3·85	3·3	37·5	8
Muscle	2·73	0·75	2·3	0·88	7·0	5·83	7·7	6·6	9·0	2
Brain	5·9	1·6	4·8	1·85	2·7	2·25	4·54	4·0	3·0	0·6
Large intestine (wall)	4·8	1·3	5·3	2·04	4·6	3·83	17·4	14·9	...	...
Small intestine (wall)	12·35	3·3	14·6	5·6	4·7	3·91	16·6	14·2	...	...
Lymph gland ...	...	...	0·8	0·3	0·9	0·75	...	...	...	...
Bone marrow ...	13·0	3·5	12·6	4·8	8·6	7·16	8·3	7·0	...	...
Heart muscle	2·5	0·7	...	...	5·4	4·5	...	...	...	...
Fat	...	...	2·3	0·8	11·1	9·25	...	...	...	...
Thyroid G.	25·0	6·8	...	...	...	...	...	...	...	...
Testicle	...	...	...	...	2·1	1·75	3·03	2·6	...	...
Lung	...	...	...	...	11·16	9·3	...	...	...	...
Stomach	...	...	...	...	2·3	1·9	...	...	...	...
Peritoneal fluid ...	...	...	...	...	...	...	...	...	14·7	3·0

Partition of quinine between corpuscles and plasma of the blood

In the experiments recorded above, samples of blood were taken up (4 c.c. usually) in a syringe containing 1 c.c. of 1 per cent. potassium oxalate. This was centrifuged at once and the quinine content of the plasma and corpuscles estimated separately.

Rabbit (1) 100 grammes of Plasma contain 2·4 mgm. quinine.
100 grammes of corpuscles contain 1·26 mgm. quinine.

$$\frac{\text{Plasma quinine}}{\text{Corpuscle quinine}} = \frac{2}{1}$$

Rabbit (2) 100 grammes of Plasma contain 0·81 mgm. quinine.
100 grammes of corpuscles contain 0·39 mgm. quinine.

$$\frac{\text{Plasma quinine}}{\text{Corpuscle quinine}} = \frac{2{\cdot}1}{1}$$

Guinea-pig 100 grammes of Plasma contain 1·98 mgm. quinine.
100 grammes of corpuscle contain 0·62 mgm. quinine.

$$\frac{\text{Plasma quinine}}{\text{Corpuscle quinine}} = \frac{3{\cdot}2}{1}$$

The blood from a case of blackwater fever gave the ratio

$$\frac{\text{Plasma quinine}}{\text{Corpuscle quinine}} = \frac{2{\cdot}2}{1}$$

In a previous paper (6), one observation on the blood of a guinea-pig gave a ratio

$$\frac{\text{Serum quinine}}{\text{Corpuscle quinine}} = \frac{3}{1}$$

ACCUMULATION OF QUININE IN EXCISED TISSUES

A. *Guinea-pig's suprarenal gland* cut in two and immersed in 10 grammes of 0·9 per cent. NaCl containing 1 mgm. quinine.

B. *Guinea-pig's suprarenal* cut in two, immersed in 15 grammes of 0·9 per cent. NaCl containing 15 mgm. quinine.

C. 0·96 *gramme of guinea-pig's spleen* immersed in 10 grammes of 0·9 per cent. NaCl containing 1 mgm. of quinine. All were kept for forty-eight hours at 35°C., and in each case the quinine content of the tissue and fluid were estimated separately.

	Grammes of tissue taken	Grammes of medium	Mgms. quinine in 100 grammes of bathing fluid		Mgms. quinine per 100 grammes tissue	Quinine concentration in tissue / Quinine concentration in Medium
			Initially	Finally		
A.	0·21	10	10	2·5	307·1	123·0
B.	0·28	15	100	18·0	2677·4	154·3
C.	0·96	10	10	2·8	77·0	22·5

It is seen that the excised tissues, and especially the suprarenal gland, under conditions where their vital processes must be reduced to a low ebb, accumulate quinine from the environment at enormous relative concentration just as they do during life, a fact which indicates that the accumulation is dependent on some specific chemical or physical affinity between quinine and one or other of the cell constituents—it is tempting to associate it with the lipoids of the cortex. To get further light on the question, attempts were made to find out whether the accumulation was mainly in the cortex or in the medulla. Glands containing large amounts of quinine, ranging from 250 to 385 mgm. per 100 grammes of tissue in the corresponding glands of the other side, were incised and immersed in Christensen's Herapathite reagent for eighteen hours at laboratory temperature. After freezing and section, neither crystalline nor (black) amorphous Herapathite could be seen in either cortex or medulla, notwithstanding the large amount of quinine present.

DESTRUCTION OF QUININE IN TISSUES

The material employed for most of these experiments was obtained in the animal experiments already described. Each tissue investigated was divided symmetrically into two approximately equal portions; the quinine of one portion was estimated at once, and that of the other estimated after incubation in 0·8 per cent. sodium fluoride solution at 35°C. As the initial concentrations of quinine differ considerably in the various tissues, the results are not strictly comparable *inter se*, but indicate merely whether quinine is destroyed or not.

	Tissue examined	Grammes tissue taken	Quinine in mgm. per 100 grammes of tissue estimated at once	Quinine in mgm. per 100 grammes of tissue after incubation	Hours of incubation	% Destruction
Expt. I.— Guinea-pig	Muscle	4·75	2·73	2·00	18	26·7
	S. Intestine	0·32	12·3	4·1	18	66·76
	Blood	3·84	3·7	3·6	18	2·7
	Spleen	0·3	22·9	22·2	18	3·0
Expt. III.— Rabbit	L. Intestine	2·71	17·4	3·0	21	83·0
	S. Intestine	0·9	16·6	2·7	21	83·6
Expt. IV.— Rabbit	Kidney	3·98	20·0	9·0	21	55·0
	Spleen	0·14	24·0	23·81	24	0·8
	Testis	1·30	2·1	2·0	26	5·0
	L. Intestine	2·53	4·6	0·0	24	100·0
	S. Intestine	2·23	2·7	2·2	24	18·5
	Muscle	1·20	7·0	4·0	24	43·0
	Marrow	0·65	8·6	8·32	24	3·3·
Guinea-pig	Kidney	2·45	85·0	80·0	18¾	6·0
	Suprarenal	0·32	1210·0	1200·0	18¾	0·8
	Muscle	3·49	9·0	6·2	17¾	31·0
	Peritoneal F.	2·41	14·7	14·7	16¾	0·0
	Pancreas	1·53	15·0	11·0	18¾	37·0
	Thyroid	10·0	1 mgm.	1 mgm.	24	0·0
	Salivary G.	7·5	1	1	24	0·0

In the following estimations, the tissues examined were pulped in a mortar and added to a solution of quinine in 0·8 per cent. NaF and incubated for varying periods at 35° C. All were fresh, but taken from various guinea-pigs.

Tissue	Grammes tissue taken	Quinine added mgm.	Found	Incubation period hours	Loss %
Lymph gland	6·3	1	0·95	63	5·0
,, ...	3·6	1	0·91	17	9·0
,, ...	5·0	1	1·0	23	0·0
Kidney	4·7	1	0·93	21	7·0
,, ...	3·6	1	0·83	21	16·6
Suprarenal	2·5	1	0·95	63	5·0
,, ...	0·76	1	0·95	23	5·0
Bone marrow	0·84	1	0·97	21	3·0
,, ...	0·69	1	0·96	21	4·0
Spleen	2·7	1	0·95	21	5·0

MAIN CONCLUSIONS CONCERNING THE ACCUMULATION AND DESTRUCTION OF QUININE IN TISSUES

Tissue	Relative accumulating power	Relative destructive power
Liver	(++)	+++++
Spleen	+++	nil
Bone marrow	++	nil
Kidney	(+++)	++
Suprarenal	+++++	nil
Muscle	++	++
Intestinal wall	(++)	++++
Pancreas	(++)	++
Lymph gland	nil	nil or slight

In column 2, tentative conclusions regarding tissues with execretory or destroying powers are indicated by brackets.

PERFUSION EXPERIMENT

A liver from a young sheep, freshly killed, was perfused for two hours at 37° C. with 2 litres of oxygenated Locke's solution containing 200 mgm. of quinine hydrochloride not acid to Congo red. After perfusion, the liver was gently squeezed. The total quantity of fluid collected was 2024 c.c.

Weight of liver before perfusion ... 978·6 grm.
" " after " ... 952·4 "

A portion of the liver was estimated for its quinine content—the total calculated for the whole liver was 18·68 mgm. quinine.

As the total perfusion fluid finally contained 36·8 mgm. of quinine, 72·26 per cent. of the quinine had been destroyed in two hours. The final concentrations of quinine in the liver and perfusion fluid, weight for weight, were approximately equal.

A weighed portion of the liver was pulped and incubated in 0·8 per cent. NaF at 35° C. for forty-five hours. The whole of its

quinine was then found to have disappeared. In the perfusion fluid similarly incubated only 8·8 per cent. of its quinine had disappeared. During the perfusion 978·6 grammes of liver destroyed 144·52 mgm. of quinine, i.e. 1 gramme of liver destroyed 0·148 mgm. of quinine (cf. Experiment A of last paper, p. 231, where 0·4 mgm. quinine was destroyed by 1 gramme of guinea-pig's liver post-mortem).

At this rate 1·7 grammes (27 grains) of quinine would have been destroyed in twenty-four hours.

OBSERVATIONS ON THE QUININE DESTROYING AGENT FOUND IN LIVER

Thermolability

10 per cent. emulsions of guinea-pig liver and ox liver in 0·9 per cent. NaCl. Boiled for three minutes on a water bath. Quinine added and the mixture allowed to stand for twenty hours at 37° C.

Tissue	Animal	Mgm. quinine added	Found	Loss%
Liver	Sheep	0·2	1·193	3·35
„	Guinea-pig ...	1·0	0·93	7·0
„	Ox	1·0	0·95	5·0
„	Guinea-pig ...	1·0	0·901	9·1

The experiments were repeated with unboiled liver extracts.

Animal	Mgm. quinine added	Found	Loss%
Sheep	0·2	0·14	30
Guinea-pig	1·0	0·47	53
Ox	1·0	0·74	26
Guinea-pig	1·0	0·58	41

It is evident that the active agent is thermolabile.

Influence of the Reaction of the Substrate

The experiments recorded below show that the enzyme acts best in neutral solution, and that alkalinity is more inhibitory than acidity.

To three flasks A, B and C, each containing 10 c.c. of a 10 per cent. emulsion of fresh guinea-pig's liver in a 0·9 per cent. NaCl and 1 mgm. of quinine, was added:—

(*a*) 10 c.c. of 0·9 % NaCl.
(*b*) 10 c.c. of 0·4 HCl.
(*c*) 10 c.c. of 0·9 % Na_2CO_8.

All were kept at 35° C. for twenty hours and the quinine then estimated.

		Mgm. Quinine added	Found	Loss %
A.	Neutral	1	0·71	28·6
B.	Acid	1	0·83	16·7
C.	Alkaline	1	0·91	9·1

The experiment was repeated with ox liver:—

		Mgm. Quinine added	Found	Loss %
A.	Neutral	1	0·62	37·5
B.	Acid	1	0·78	21·4
C.	Alkaline	1	0·89	10·7

Repeated with dried ox liver:—

		Mgm. Quinine added	Found	Loss %
A.	Neutral	1	0·77	23·0
B.	Acid	1	0·87	13·0
C.	Alkaline	1	0·91	9·1

Repeated with guinea-pig liver:—

A. 10 c.c. of 10 % extract + 1 mgm. Q. + 10 c.c. 9 % NaCl.
B. 10 c.c. of 10 % extract + 1 mgm. Q. + 10 c.c. $\frac{N}{10}$ H_2SO_4.
C. 10 c.c. of 10 % extract + 1 mgm. Q. + 10 c.c. $\frac{N}{10}$ NaOH.

	Reaction	Mgm. Quinine added	Found	Loss %
A.	Neutral	1	0·62	37·5
B.	Acid	1	0·74	26·7
C.	Alkaline	1	0·83	16·7

The Influence of Oxygen

There was some indication in the paper by Ramsden, Lipkin and Whitley (1918) (p. 231), that a free supply of air promoted the destruction of quinine by liver pulp.

The following experiments show that oxygen is absolutely essential. 10 c.c. of 50 per cent. fresh liver extract in 0·8 per cent. NaF solution was incubated for twenty hours at 35° C. In Case A, a current of air was gently drawn over the mixture without mechanical disturbance of the surface; in Case B, no air was drawn over the mixture:—

	Mgm. Quinine added	Found	Loss %
A.	1	0·43	57·0
B.	1	0·62	38·0
This was repeated.			
A.	1	0·40	60·0
B.	1	0·61	39·0

A. 20 c.c. of fresh guinea-pig's liver extract in 0·9 per cent. NaCl + 1 mgm. quinine in solution mixed, and a current of air drawn over the mixture at laboratory temperature for twenty-one hours.

B. 20 c.c. of the same liver extract placed in a flask and 1 mgm. of quinine in solution in a tube inside the flask. The flask was exhausted and filled with hydrogen. The quinine was then tilted into the liver extract and the sealed flask allowed to stand for twenty-one hours.

	Mgm. Quinine added	Found	Loss %
A.	1	0·4	60·0
B.	1	0·83	16·7

As complete removal of oxygen was uncertain, the experiment was repeated with extra precautions.

A. 10 c.c. of a 0·50 per cent. extract of guinea-pig's liver in 0·8 per cent. + 5 drops of ammonium bisulphide solution + 1 mgm. quinine in solution. The flask was exhausted and then filled with oxygen and sealed.

B. 10 c.c. of 0·8 per cent. NaF solution + 5 drops of strong ammonium bisulphide solution + 1 mgm. of quinine in solution.

C. 10 c.c. of same liver extract + 5 drops of strong ammonium sulphide solution, 1 mgm. quinine in solution placed in a small test-tube inside the flask. The flask was exhausted and then filled with hydrogen freed from all traces of oxygen by passage through a red hot copper tube. This was repeated three times before the final sealing.

The quinine solution was then tipped into the liver extract. All the flasks were allowed to stand for twenty hours at laboratory temperature, and were heated to 100° C. for two minutes before they were opened.

	Mgm. Quinine added	Found	Loss %
A.	1	0·23	77·0
B.	1	0·97	3·0
C.	1	0·93	6·2

The losses in B and C have no significance, as they are attributable to experimental error.

Influence of Hydrogen Peroxide on the Ferment

A. 10 c.c. of fresh extract of guinea-pig's liver in 9 per cent. NaCl + 10 c.c. of distilled water and 1 mgm. quinine.

B. 10 c.c. of same liver extract + 10 c.c. of neutral H_2O_2 + 1 mgm. quinine.

C. 10 c.c. of same liver extract boiled and cooled + 10 c.c. of carefully neutralised H_2O_2 + 1 mgm. quinine.

	Mgm. Quinine added	Found	Loss %
A.	1	0·58	41·2
B.	1	0·76	23·1
C.	1	0·95	5·0

Evidently hydrogen peroxide is detrimental. In the control experiment C, the hydrogen peroxide had not affected the quinine.

Repeating the experiment with bile salts instead of hydrogen peroxide showed that they had no effect on the destruction.

RAPIDITY OF QUININE DESTRUCTION

A series of flasks containing 10 c.c. of 25 per cent. guinea-pig liver extract in 0·8 per cent. NaF + 1 mgm. quinine. The quinine content of the flasks was estimated after known intervals of time.

Mgm. quinine added	Found	Time of exposure quinine in the liver extract	Loss %	% of total destruction
1	0·83	1 minute	16·67	32·56
1	0·68	15 „	31·04	60·62
1	0·62	30 „	37·5	73·24
1	0·58	60 „	41·18	80·43
1	0·55	3 hours	44·4	86·72
1	0·52	24 „	47·37	92·54
1	0·48	48 „	51·2	100·00

It will be seen that the rate of destruction is at first considerable but soon becomes slow—in the first fifteen minutes 37 per cent. quinine was destroyed, in the second 6·8 per cent., and in the next thirty minutes only 3·7 per cent.

PURIFICATION OF THE ENZYME

It had already been shown that 50 per cent. alcohol extracts the active principle from liver. It is possible by a process of fractional precipitation with alcohol to obtain the ferment in purer form. 500 grammes of minced liver were extracted with 25 per cent. alcohol and filtered till clear. The filtrate was red with haemoglobin. Alcohol was then added until by volume 36 per cent. was present, and the mixture was filtered. By further successive additions of alcohol and filtering after each addition, solutions were obtained with 48·8 per cent., 59 per cent., 68·8 per cent., 83 per cent. and 92 per cent., respectively.

A 20 c.c. sample of each filtrate was incubated for twenty-one hours with 1 mgm. quinine, and the destruction of quinine estimated with the following results:—

Percentage volume of alcohol in filtrate	Mgm. quinine added	Found	Loss
25·0	1	0·62	37·8
36·0	1	0·64	35·5
48·8	1	0·66	33·3
59·0	1	0·77	23·0
68·8	1	0·97	0·0
83·0	1	0·96	4·0
92·0	1	0·97	5·0
92·0	0	0·0	

It is clear that either the active agent was completely precipitated when the alcohol reached 68·8 per cent. and upwards by volume, and that up to 59 per cent. very little was precipitated, or that more than 59 per cent. inhibits its activity.

PREPARATION OF ENZYME BY ALCOHOL PRECIPITATION

415 grm. of minced guinea-pig liver were extracted with 50 per cent. alcohol and allowed to stand for two hours and then filtered clear.

To the straw-coloured filtrate absolute alcohol was added until 70 per cent. was present. The precipitate was allowed to stand for four hours, then filtered off, and dried in a dessicator.

The product was a whitish-yellow powder which gave no purpurogallin with pyrogallic acid, but contained catalase and haemoglobin (guiacum reaction). 0·4 grm. of the powder was shaken with 20 c.c. of water and filtered—the filtrate was colourless, faintly opalescent, and contained protein in small amount. 10 c.c. of it was incubated with 1 mgm. quinine for eighteen hours—50 per cent. of the quinine was destroyed.

Two weeks later the experiment was repeated : only 37·5 per cent of the quinine was destroyed—apparently the powder deteriorates on standing. An attempt was made to purify the enzyme after the principle found effective for fibrin ferment by Schmidt. 250 grms. of minced fresh guinea-pig liver were extracted with absolute alcohol for twenty-four hours, and then shaken with ether, dried in a

dessicator and ground to a fine powder. The powder, when shaken up with distilled water and filtered gave a clear solution poor in protein. 1 grm. of the powder was incubated at 35° C. with 1 mgm. quinine + 20 c.c. of distilled water for twenty-one hours. Its action was feeble—only 26 per cent. of the quinine was destroyed.

1 grm. of dried crude powdered ox liver, tested similarly, destroyed nearly as much, namely 17 per cent. It was evident that by exposure to absolute alcohol most of the enzyme had been inactivated.

THE PRODUCTS OF QUININE DESTRUCTION BY LIVER

Having found that the liver destroyed quinine only in the presence of oxygen, an attempt was made to identify the products formed. Quitenine ($C_{18}H_{22}N_2O_2COOH$), a well-known oxidation product of quinine ($C_{18}H_{22}N_2O_2CH : CH_2$), was an obvious possibility, since Kerner (1870) had described its occurrence in the urine of patients taking quinine, and although this had been denied by Merkel (1902) and by Giemsa and Schaumann (1907), Dr. Nierenstein (1919) had informed me that he had found it in the urine in the earliest stages of quinine excretion.

Professor Ramsden kindly prepared some pure quitenine by Skraup's (1880) method, and together we studied some of its properties. In addition to the properties assigned to it by Skraup and Nierenstein, we find that although it gives with bromine water and ammonia an apparent Thalleioquin reaction, the initial colour is much bluer though it goes green on standing, and that, unlike the quinine pigment, this blue compound cannot be extracted by shaking with chloroform. This insolubility in chloroform furnishes a useful means of distinguishing it from quinine.

Crystals of quitenine from alcoholic solution are transparent, and show varying 'relief' when examined through a rotating Nicol's prism. When heated in water the crystals fragment, and become opaque at temperature well below 80° C. Quitenine picrate is insoluble in ether. Tanret's reagent is not nearly so delicate a precipitant for quitenine as for quinine, although, as in the case of quinine, the presence of much ammonium sulphate greatly enhances its delicacy. With Christensen's reagent some darkening occurs, but no polarising crystals are obtainable.

EXPERIMENT 1. $1\frac{1}{4}$ lbs. of freshly minced sheep liver in 200 c.c. 0·9 per cent. NaCl with 5 grms. of quinine dissolved with the aid of a minimum of HCl, was incubated at 35° C. for eighteen hours. The suspension was raised to 100° C. for a few moments, 'defaecated' and filtered. The filtrate was saturated with picric acid and filtered clear. The precipitate was suspended in 100 c.c. of 1·0 per cent. HCl, and the picric acid was completely extracted by shaking with ether. The acid aqueous layer was rendered alkaline with NaOH, and the quinine then removed by ether. A current of CO_2 was passed through the alkaline aqueous liquid, and the flocculent precipitate formed was dissolved in boiling alcohol water mixture (two parts of water to one of alcohol). The clear filtrate obtained on cooling deposited clear colourless crystals which gave all the reactions and possessed all the properties of quitenine.

The anhydrous crystals melted at 233° C. The total amount of quitenine obtained weighed about 500 mgm.

EXPERIMENT 2. 800 grms. of guinea-pig liver, finely minced, suspended in 200 c.c. 0·8 per cent. NaF containing 1 grm. of quinine in solution, incubated for eighteen hours at 35° C.

Traces of quitenine were found.

EXPERIMENT 3. 350 grms. of minced guinea-pig's liver in 0·8 per cent. NaF + 4 grms. of dissolved quinine in it, were incubated for twenty hours at 35° C.

Following the same procedure as in Experiment 1, no precipitate was obtained with the CO_2. The solution was evaporated to dryness and the residue extracted with absolute alcohol. No quitenine, but an interesting and apparently new quinine derivative was obtained as an amorphous residue soluble in alcohol, water, acids and alkalies, and giving with bromine water and ammonia a green pigment which was not extracted by chloroform. Its picrate was soluble in ether. With Christensen's Herapathite reagent on a slide it gave colourless long needle crystals. It gave a strong yellow turbidity with Tanret's reagent.

EXPERIMENT 4. 375 grms. of guinea-pig liver incubated for forty-eight hours to 35° C. with 2·5 grms. of quinine HCl—quitenine was found in small quantity, only 24 mgm. (anhydrous) being obtained.

QUITENINE IN URINE

To test the influence of quitenine on the malarial parasite, a patient, aged 23, was given, on the 11th of April, four 5-grain doses of quitenine hydrochloride in solution orally, six such doses on the 12th, and two 5-grain doses on the 13th. The patient had typical malarial rigors from the 10th to the 15th, inclusive. The quitenine had no effect on the clinical symptoms or on the presence of parasites in the blood. On the 15th, the patient was given 45 grains of quinine—no further rigor occurred, and no malarial parasites could be found in the blood on the 17th.

The urine collected in the twenty-four hours between the 12th and 13th measured 1500 c.c.; 750 c.c. of this was examined for quitenine by the method already described, by which I found I could easily detect as little as 3 mgms. in 100 c.c. of urine, but no quitenine whatever was found. No quitenine could be found in a later sample of urine. The faeces were not investigated, but it may be safely assumed, in view of Kerner's (2) observations on animals, that most of the quitenine had been absorbed. It would seem that in the doses given, quitenine is not only itself ineffective but is broken up into therapeutically ineffective compounds, since little, if any, appeared in the urine.

NOTE ON A CASE OF BLACKWATER FEVER

Three 15-grain doses of quinine sulphate in solution were given orally to a man, aged 23, who had been suffering from malaria for two years, and whose blood contained malignant tertian parasites. Nine hours after the last dose of quinine, the patient vomited, had a rigor, passed dark urine and became jaundiced.

A sample of urine passed three hours after the onset of the rigor was dark red in colour, and contained oxyhaemoglobin, methaemoglobin, urobilin in great excess, albumen in fair amount, and débris consisting of cellular and amorphous matter and haematin.

A sample of blood taken sixteen hours after the onset of blackwater was found to contain 0·67 mgm. of quinine per 100 grms. of blood (7·1 mgm. quinine per litre of blood).

$$\frac{\text{Plasma quinine concentration}}{\text{Corpuscle quinine concentration}} = \frac{2.2}{1}$$

The blood was centrifuged immediately, the serum was deep reddish brown in colour and gave a strong spectrum of mixed oxyhaemoglobin and methaemoglobin. Urobilin was present.

A sample of urine passed ten minutes after taking the blood sample was estimated to contain 35 mgm. of quinine per litre of urine.

$$\frac{\text{Urine quinine concentration}}{\text{Blood quinine concentration}} = \frac{8.73}{1}$$

This remarkably low ratio has been noticed before in blackwater fever (cf. Ramsden, Lipkin and Whitley (1918), page 246).

Haemoglobin was found in the urine until sixty hours, and quinine until sixty-four hours after the last dose of quinine.

EXAMINATION OF THE FAECES

Twenty-six grms. of moist faeces passed eighteen hours after the onset of blackwater was suspended in 1 per cent. H_2SO_4 and saturated with $(NH_4)_2SO_4$, 50 c.c. of absolute alcohol added, and the whole mixture boiled and filtered. The residue was washed with more hot alcohol. The combined filtrates were shaken up with ether. The ether-alcohol layer which separated was very dark brown, almost black in colour. This was pipetted off into another vessel and allowed to stand. A large quantity of blackish brown material separated out.

A few drops of this ether extract poured on to a filter paper turned gradually from deep brown to green, yellow and red (a play of colours due apparently to oxidation by the air).

The black precipitate from the ether was washed free from stercobilin with ether and dried. It was insoluble in water, acids, alkalies, chloroform, benzene, amyl alcohol, toluene, ether, but very freely soluble in alcohol. The alcoholic solution showed no spectral absorption bands, and was dark brown in colour. It turned purple on standing, especially if the alcoholic solution was acidified with hydrochloric acid. This purple pigment proved to be cholecyanin—the acid solution showing three well marked absorption bands. One thin band between the C and D lines, another broader band on the green side of the D line, and a third very broad band between b and F lines.

On rendering alkaline with ammonia the purple colour was replaced by yellow, showing only one feeble absorption band on the D line; the purple colour reappeared on acidification.

The purple pigment could be extracted in ether, chloroform, toluene or benzene, giving solutions nearly pure blue in colour.

It seems highly probable that the initial black substance, certainly not itself cholecyanin although so easily converted into it, is a cholecyaninogen. None could be found in the urine.

NOTE ON THE THALLEIOQUIN REACTION

A great drawback to this very useful test for the quinine group of alkaloids is its lack of sensitiveness.

The success of the test depends, among other factors, on—

1. The concentration of the quinine in the solution to be tested.
2. The avoiding of excess of bromine.

Ramsden and Lipkin (1918*a*) found that, using 10 c.c. of solution, the least concentration of quinine certain to give a positive reaction with suitable precautions was 1 in 400,000, and that 0·25 mgm. of quinine could thus be detected.

By the following procedure, in which quinine is first isolated as described for the herapath test and then dealt with in strong solution, 0·004 mgm. of quinine can easily be detected in 40 c.c. of water (1 in 10,000,000):—

1. Dissolve 5 grms. of $(NH_4)_2SO_4$ in every 10 c.c. of the quinine solution, alkalise with ammonia and extract the quinine by shaking with two successive lots of purified ether, transferring each lot as it separates to a small silica crucible in a water bath. Aspiration of air from its interior greatly accelerates drying.

2. Dissolve the residue when quite dry in a minimum of ether squirted repeatedly down the side of the crucible by means of a small teat pipette.

3. Bring the ether solution in minimum drops on to a warm microscope slide, in such a way that the residue left by its evaporation is spread over a minimal area.

4. Cover the area of the residue with a very small drop of 0·4 per cent. HCl, now add a small drop (1/30 c.c.) of weak bromine

water and immediately after a small drop of strong ammonia. A green colour is obtained at once. On addition of a drop of strong H_2SO_4, the green colour turns to a distinct red.

I have found that the following modification of the thalleioquin test, although its rationale is by no means clear, serves to distinguish quinotoxin from quinine. To 5 c.c. of the solution containing quinine or quinotoxin add a small drop of 0·5 per cent. Congo red in water. Add bromine water till the blue colour is discharged and replaced by a yellow colour. Add ammonia at once and extract the pigment with chloroform. Quinine gives a green, quinotoxin a red colour, both extracted by chloroform, but the red much more rapidly. One part of quinotoxin in twenty parts of quinine can be detected by this means.

DISCUSSION

(Written jointly with Professor Ramsden)

One of the main objects of the work has been to get fresh light on the question, how it is that quinine, although almost always successful in causing the disappearance of malarial parasites from the circulating blood and effecting a temporary cure, nevertheless almost always fails to obviate eventual relapse. That this failure is not due to any lack of quinine in the general blood-stream, at least up to almost intolerable amounts throughout a period of many hours, has been shown by Ramsden, Lipkin and Whitley (1918*b*) in collaboration with Professors Stephens and Yorke.

Assuming for the moment that quinine is directly parasiticidal, and also that its metabolites may be neglected, various explanations may be suggested for this failure:—

1. The existence of quinine-resistant parasites or of a specially resistant phase in their life history.
2. Parasites may find safety outside the blood, either in other body-fluids or inside cells free from quinine.
3. Parasites may find 'backwaters' maintained free from quinine inside the blood vascular system.

No conclusive evidence can be adduced for any of these possibilities, but the data showing the quinine-destroying powers and quinine content of the various tissues have obvious bearings on

the last two of them, although subject to the disadvantages that post-mortem destruction is not a very safe guide to destruction in vivo and that the average quinine-content of a tissue is not necessarily representative of all the cells of that tissue.

It should also be pointed out that so far as extracellular parasites are concerned the data showing the quinine-content of various tissues, although of much interest in relation to absorption and storage of quinine and the physico-chemical conditions inside the cells, do not justify any conclusion as to the *effective* concentration of the drug inside the cell—since much of it might be harmless to the parasite if precipitated or absorbed by the colloids or dissolved in the lipoids of the host cell, or in some non-toxic chemical combination.

As regards the hypothetical places of safety in 'backwaters' inside the blood-vascular system, it may be pointed out that if quinine alone is parasiticidal, they must satisfy the following requirements:—

(*a*) Parasites must be able to remain in them throughout such periods as the concentration of quinine in the general blood stream is actually maintained at a toxic level.

(*b*) They must have a blood supply such that even in many hours only a small proportion of the total blood passes into them—otherwise quinine would not be present in quantity in the general blood-stream so many hours after a dose, as in fact it is (cf. (1918*b*) page 241).

(*c*) They must be surrounded by tissues which remove quinine from them at least as rapidly as fresh supplies are brought up.

Of the powers of the various tissues to remove quinine from the blood, the data obtained give only imperfect evidence—this power must depend not only on the ability of the tissue to destroy or to excrete the drug but also on its storage capacity, and in the case of those tissues which destroy or excrete quinine this can only be dimly guessed at.

It is, however, clear that the tissues mainly responsible for the destruction of quinine are the liver, intestinal wall, muscles and kidneys, and that the blood and spleen have little or no such power. It is clear, also, that each of the tissues examined, except the lymph

glands and red blood corpuscles, store much more quinine weight for weight than does the blood or the blood plasma, and that this storage capacity is especially high in the suprarenals, spleen and kidney.

The considerable variations in relative storage capacity of the different tissues in different experiments (cf. suprarenals with intravenous and intraperitoneal injections) may possibly depend on variations in the physiological condition of the tissues and in their blood supply.

Failing exhaustive anatomical knowledge of the 'backwaters' which may exist in various regions of the blood vascular system, and therefore considering only the bone-marrow and the spleen in which their existence is beyond question, it is clear that since neither of these tissues is capable of destroying quinine post-mortem, such quinine-removing power as they possess probably depends solely on their storage power, and of this nothing is known except that it is greater than in many other tissues and might conceivably be adequate for maintaining quinine-free 'backwaters' (especially, perhaps, in the bone-marrow) provided the access of fresh blood is sufficiently slow.

The lymph-glands are remarkable as being the only tissue in which the quinine concentration is consistently less than that in the blood. Whether this is due to poor blood supply or to relative impermeability to quinine is uncertain. That it is probably not due to quinine destruction is indicated by the absence of such in the one post-mortem experiment made.

If the parasiticidal effect of quinine in malaria be not due to quinine itself but to one or other of its metabolites, other possibilities arise for the survival of the parasite, e.g. they might be safe in *any* backwater if the toxic concentration of the effective metabolite is maintained only in the blood of the quinine-destroying tissues. Until the existence of such metabolite has been actually demonstrated, further discussion would however scarcely be profitable.

That the active metabolite, if it exists, is neither quitenine nor any of its metabolite products, is strongly indicated by the observation that this substance had no therapeutic value in malaria and was not excreted as such in the urine, and by Kerner's (2) observation that it

has little or no toxicity for animals although readily absorbed by them. It would appear that with the oxidation in the liver of the 'vinyl' group of quinine (R.CH : CH_2) into the carboxyl group of quitenine (R.COOH) all therapeutic activity is lost.

Whether the whole of the quinine which is metabolised in the body is converted into quitenine can only be proved by quantitative work. If it is, the only metabolite of quinine which could conceivably be therapeutically active would be the intermediate aldehyde R.CHO. The unknown substance found in 'hepatised quinine' (Experiment 3, p. 168) is of special interest in this connection, and deserves further investigation.

SUMMARY

1. Evidence is given bearing on the possibility that there exist in the blood-vascular system, regions kept almost free from quinine throughout a period of quinine medication.

2. The quinine content of tissues has been more extensively studied. Accumulation at much higher concentration in most tissues than in the blood is confirmed.

3. The suprarenal body is pre-eminent in this respect, although not so markedly with intramuscular as with intraperitoneal injections. Fairly large accumulations may occur also in the spleen and kidney: the lymph glands contain much less quinine than the surrounding blood.

4. The liver, kidney, muscle, intestinal wall, and probably pancreas, have considerable power to destroy quinine post-mortem, and, therefore, presumably during life. The blood, spleen, suprarenal bodies, bone-marrow, lymphatic, salivary, and thyroid glands have little or no such power.

5. The quinine-destroying agent extracted from the liver is thermolabile, inactivated at 100° C. and acts best in neutral media. Its action is rapid at first, but soon falls off. It does not act at all in the absence of oxygen, and is hindered by hydrogen peroxide. It can be crudely 'purified' by fractional precipitation with alcohol.

6. Quitenine is formed by the action of liver pulp on quinine.

7. Quitenine given by the mouth, in ample doses to a malarial

patient, was therapeutically inert. As none appeared in the urine, this is probably true also of its metabolites.

8. In the faeces of a case of blackwater fever, a brown pigment is described which, although not itself cholecyanin, readily yields this body.

9. New tests for quitenine and quinotoxin are described.

10. By an improved procedure, the thalleioquin test is rendered capable of detecting easily 0·004 mgm. of quinine.

In conclusion, I wish to record my thanks to Professor W. Ramsden for the valuable help and advice he unstintingly gave me during this work, and for his collaboration in discussing the results of the investigation.

I am indebted also to Professors J. W. W. Stephens and W. Yorke for help with clinical experiments and animal injections.

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